CHARACTERIZATION OF MICELLAR MOBILE PHASES FOR REVERSED PHASE LIQUID CHROMATOGRAPHY

BY

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1983

To my mother, Christa Landy

ACKNOWLEDGEMENTS

Gloria B. DiMonte, oh love of my life...four and one-half years ago, with your consent, I left for the University of Florida. The years would have seemed much longer if not for your love, constant encouragement, and faith in me, our vacations together, and Ma Bell. We didn't plan it this way, but thanks for waiting, and some day we'll be together for more than two weeks.

To my friends in Dr. Dorsey's group, I appreciate your companionship, admire your patience with me, and will miss all of you.

To Laura Griggs, thank you, your speed, expert typing, and editorial skills were invaluable.

Finally, to Dr. John G. Dorsey, I hope I can adequately express my gratitiude to you. Your contributions to this dissertation and other projects have been immeasurable and manifest. You have given excellent advice and guidance throughout the years, including the traumatic period of job hunting from resume to business contacts. Despite your serious deficiency (an unexplainable fascination with the Atlanta Braves), you have many fine traits, including infinite patience, humor, wit, confidence building, and generosity; and I thank you for sharing them with me. Your

friendship over the years can easily be correlated with my sanity. I hope to keep our association a permanent one.

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Abstract of Dissertation Presented to the Graduate Council of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

CHARACTERIZATION OF MICELLAR MOBILE PHASES FOR REVERSED PHASE LIQUID CHROMATOGRAPHY

By

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December 1983

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Major Department: Chemistry

The use of micellar mobile phases for reversed phase liquid chromatography with emphasis on efficiency optimization, physical studies, selectivity, and gradient elution capabilities is examined, and whenever relevent, compared to traditional hydroorganic mobile phases. Surfactants self-aggregate into larger units called micelles, which provide a hydrophobic site for interaction with the solute in the mobile phase and can be used in place of an organic modifier. Certain advantages exist for micellar mobile phases including unique selectivities, economic considerations, purity, and safety.

Purely aqueous micellar mobile phases can provide unique selectivities in liquid chromatography, however are

very inefficient in comparison to traditional hydroorganic mobile phases. This inefficiency is shown to arise from slow mass transfer, which comes from poor wetting of the stationary phase. Low concentrations of organic modifiers are useful for modifying the surface of the stationary phase and providing the wetting needed for good mass transfer. Elevated temperatures are also shown to be useful in overcoming the higher viscosity of micellar mobile phases and thereby serving to improve peak shape.

One of the most basic calculations of any micellar solution is a term called the critical micelle concentration (CMC), which describes the concentration when micellization occurs. The CMC's of 3 surfactants are determined chromatographically, and other physical studies of micelles are described. Heats of transfer are calculated for both micellar and non-micellar mobile phases which demonstrates the ability of the micelle to compete with the stationary phase for the solute. Electrolytes may affect the polarity of micelles by changing the micellar structure. Liquid chromatography is suggested as a sensitive technique to monitor structural changes, although these results are as of yet inconclusive.

The capacity factor and selectivity were chosen to express the retention behavior of four non-polar solutes for each surfactant studied. The combined mechanisms of ion-pairing and partitioning into micelles describes the electrostatic effects of ionic solutes with ionic surfactants above

the CMC. Gradient elution for micellar mobile phases is shown to be feasible and much improved as compared to traditional hydroorganic mobile phases due to fast reequilibration times.

CHAPTER I

INTRODUCTION

Surfactant Background

A surface active agent, or surfactant, is an amphiphilic molecule composed of a polar hydrophilic head group attached to a long nonpolar hydrophobic tail. As the name implies, surface active agents adsorb onto surfaces or interfaces, altering the surface of two immiscible phases. Surfactants have been used for such purposes as detergents, toiletries, pharmaceuticals, tertiary oil recovery, flotation agents, dispersants, germicides, and corrosion inhibitors [1-4].

Above a certain concentration, termed the critical micelle concentration (CMC), the surfactant molecules tend to dynamically self-aggregate forming symmetrical, often spherical structures called micelles. In the case of aqueous solutions, the polar head groups project into the aqueous solution, surrounded by counterions, if any, and interact electrostatically with water, while the hydrocarbon tails directed inward form a liquid-like core. A Dill-Flory representation of a micelle is shown in Figure 1 [5]. The general size and geometry of each micelle in a particular system is controlled by a certain number of surfactant molecules termed the aggregation number.

Figure 1. Representation of the Dill-Flory micelle.

From equations (1) and (2),

$$[\text{Surfactant}_{\text{Total}}] - \text{CMC} = [\text{Surfactant}_{\text{As Micelles}}] \quad (1)$$

where

$$\text{CMC} \approx [\text{Surfactant}_{\text{As Free Monomer}}] \quad (2)$$

it can be seen that the CMC is the constant concentration of free monomers at any total concentration of surfactant after micellization has occured. Because micelles are not very surface active and essentially only exist above the CMC, the physical properties of micellar solutions are very different from those of surfactant solutions below the CMC.

The most significant physical property of micellar solutions is related to their solubilization of otherwise water-insoluble compounds [6,7]. This solubility effect has led to the study of micellar solutions in various areas of chemistry. Biochemists relate basic structural similarities of membranes and micelles to the transport, absorption and solubilization of many important physiological molecules [3,8]. There have been many studies involving micellar media and their effects on the rates of inorganic and organic reactions [9-11]. Micellar catalysis is very dependent on the particular system involved [3].

Micellar systems have been used to advantage also in many areas of analytical chemistry for such problems as solubility of reagents and analytes, slow kinetics of reactions, sensitivity, selectivity, and many others. Hinze [6] gives an excellent review of the uses of surfactants in

analytical chemistry. Micellar systems have been developed for ultraviolet-visible absorption, atomic absorption, and luminescent methods of analysis. Fundamental electroanalytical applications of micelles include polarography and potentiometric titrations. There are reports of surfactant and micellar systems used for analytical extractions and separations, but until recently there had been no chromatographic applications of these systems.

Liquid Chromatography and Micelles

Liquid Chromatography (LC) is a very powerful technique for the separation of complex mixtures. In addition to the same compounds amenable to the long established technique of gas chromatography (GC), LC is capable of separating thermally unstable and nonvolatile compounds. The popularity of modern liquid chromatography then continues to increase. Indeed, in a recent survey, use of liquid chromatographs led all other analytical instruments in growth for nine consecutive years [12]. Of the 2.2 billion dollars in 1983 spent in worldwide sales of analytical instrumentation, approximately 300 million will be spent on high performance liquid chromatographs, with increases in sales projected at 25% per year in current dollars for the next several years [13]. Most of today's LC work utilizes bonded, reversed-phase microparticulate columns; their use was conservatively estimated in 1980 to include 65% of all LC separations [14].

One of the reasons for the popularity of this technique is the unique selectivities that can be generated in the mobile phase by the addition of chemical modifiers. Retention in reversed-phase LC is dominated by solvent-solute interactions, with stationary phase-solute interactions making secondary contributions. The key to separation is then to be able to change the solvent-solute interactions in such a way as to shift the retention of any overlapping compounds. In some cases, the selectivity of the chromatographic system can be modified by simply changing the eluent strength or ratio of the hydroorganic mobile phase. Most often, however, changes in selectivity of the chromatographic system reached in this fashion are accompanied by concomitant changes in the retention of all sample components, and an appreciable change in relative retention is achieved only with capacity factors, k's, that fall outside the practical range of $0.3 < k' < 5$. It is desirable to find ways to change the selectivity of the chromatographic system for closely eluting sample components without drastic changes in the eluent strength as a whole.

One way that this has been done, particularly for ionogenic solutions, is by the addition of low concentrations of ionic surface active agents having an opposite charge to the solute. The surfactant will then coulombically interact with the solute and drastically alter its retention characteristics. Because of the nature of the surfactant, this technique was dubbed "soap chromatography" [15] and is now more commonly known as ion-pair chromatography.

Until recently, only low concentrations of surfactant were used, and investigators intentionally stayed below critical micelle concentrations. The pioneering work of Armstrong and Terrill demonstrated the usefulness of reversed-phase mobile phases containing surfactants above the critical micelle concentration in thin-layer chromatography [16]. Later with Henry, Armstrong applied this technique to reversed-phase high performance liquid chromatography (HPLC) [17]. They showed that the micelle can provide a hydrophobic site for interaction with the solute in the mobile phase and can be used in place of an organic modifier. A theoretical basis involving three partition coefficients to explain the separation mechanism has been shown [18]. Armstrong has also used cyclodextrins in addition to micelles with similar results, and it appears he prefers to use the generalized term of pseudophase liquid chromatography (PLC) to include both types of modifiers. Since then, other reports have appeared [19-23], and certain advantages have been shown, including the selectivity of the micellar interaction and economy of operating expense when compared to expensive chromatographic grade organic solvents. Armstrong et al. [21] and Weinberger et al. [22] have also shown that micellar mobile phases can lead to increased selectivity of detection through the use of micelle-stabilized room-temperature phosphorescence.

"Characterization of Micellar Mobile Phases in Reversed Phase Liquid Chromatography" is a further study of micellar

mobile phases in what has recently been called a "fledgling" technique by Cline Love, one of its leading proponents [23]. Chapter II describes the experimental set-up: the LC system, reagents and solutes, and procedure. Before micellar mobile phases can be accepted as an alternative to traditional hydroorganic mobile phases, they must attain at least similar efficiencies. Chapter III deals with the inefficiency of purely aqueous micellar mobile phases and a method for optimizing efficiency. Chapter IV characterizes the micellar mobile phases in a variety of ways. The charge of the polar head group (anionic, cationic, zwitterionic or nonionic) is often used to classify a surfactant, although other classifications are possible (branching vs. straight chain tails, pure hydrocarbon vs. fluorinated hydrocarbon tails, chain lengths, counterions, etc.). The surfactants studied in detail were sodium dodecylsulfate (anionic), cetyltrimethylammonium bromide (cationic), and polyoxyethylene lauryl ether (nonionic). Critical micelle concentrations were determined for each surfactant using a chromatographic method. Thermodynamic studies were done to examine the attraction of solutes to the micelle. Physical studies of micelles were attempted to examine electrolyte effects on micellar structure. Also, retention related parameter comparisons for both nonionic and ionic solutes of all three surfactants were made and contrasted to a traditional hydroorganic mobile phase. Finally, the feasibility of micellar gradient elution is shown. Chapter V includes a conclusion section and suggestions for future work.

CHAPTER II

EXPERIMENTAL

Liquid Chromatography System

An Altex (Berkeley, California) model 322 gradient liquid chromatograph incorporating model 100A dual reciprocating pumps, model 210 sample injection valve with five and twenty microliter loops, and a model 153 UV detector (fixed at 254 nm) with an 8 μL flow cell was used. The time constant of the detector was 1.1 sec, and the extra-column volume of the system was calculated to be 60 μL.

For the efficiency studies, both an Alltech (Deerfield, Illinois) 5 μm C_{18} and Altex ODS Ultrasphere columns (4.6 × 250 mm) were used. The selectivity studies involved strictly Altex ODS Ultrasphere columns of both 15 and 25 cm lengths. To avoid such problems as precipitation, incomplete removal of adsorbed surfactant from the stationary phase, and possible mixed mechanisms, a separate column was dedicated for each different surfactant mobile phase. An Alltech 10 μm cyano column (4.6 × 250 mm) and a C1 IBM (Danbury, Connecticut) HPLC Mini column (4.5 × 50 mm, 5 μm) were used to examine the effects of salt on the structure of micelles.

A dry handpacked 10 cm precolumn of 25-40 μm silica (Macherey-Nagel and Co., Düren, Germany) was placed between the dynamic mixer and injector. It is believed that this significantly prolongs the lifetime of the analytical columns by saturating the analytical column with soluble silicates as the use of surfactant appeared to dissolve the silica backbone of the analytical column.

Both the analytical column and precolumn were thermostated using water jackets and a Techne TE-7 circulator (Cambridge, England) as seen in Figure 2. Thermostating of the precolumn served to pre-equilibrate the temperature of the mobile phase to that of the analytical column avoiding any possibility of thermal gradients developing across the analytical column.

Reagents

Mobile Phase

Surfactants were dissolved in one or more of the following: HPLC grade methanol and acetonitrile (Fisher Scientific, Fair Lawn, New Jersey), reagent grade 1-propanol (Fisher Scientific), U.S.P. ethanol (U.S. Industrial Chemicals Co., New York, New York), and deionized water purified using a Barnstead Nanopure system (Boston, Massachusetts) and irradiated by UV light to remove any trace organics. The surfactants used were HPLC grade sodium dodecyl sulfate (SDS) from Fisher Scientific and Fluka AG (Buchs, Switzerland), cetyltrimethylammonium bromide (CTAB) from Fluka AG

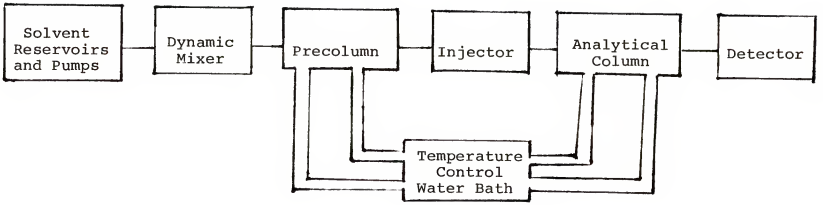


Figure 2. Thermostated LC system.

and polyoxyethylene lauryl ether (Brij-35) also from Fluka AG. These were used as received.

Solutes

The solutes were dissolved in water or methanol using concentrations in the low ppm. The reagent grade solutes, used as received, were acetophenone, aniline, nitrobenzene, ethylbenzene, and sodium chloride (all Fisher Scientific); and phenol, benzene, toluene, anthracene, and sodium benzoate (all Mallinckrodt, Paris, Kentucky).

Procedure

The appropriate weight of surfactant was dissolved in water or water-organic modifier and the solution then filtered through a 0.45 μm Nylon-66 membrane filter (Rainin Instruments, Woburn, Massachusetts). All capacity factor and plate count values are averages of at least duplicate determinations.

CHAPTER III

EFFICIENCY OPTIMIZATION

"As evidenced by several broad peaks in the foregoing examples, the efficiency of micellar chromatography is somewhat less than that of conventional reversed-phase chromatography" [23]. The above quote was taken from a November 1982 paper that focused on the selectivities of micellar mobile phases. In fact, a major drawback in all published reports is a loss of efficiency when compared to traditional hydroorganic mobile phases. If the use of micellar mobile phases is ever to be widely accepted as a viable technique, the efficiency achieved must at least approach that of conventional reversed-phase LC. This chapter discusses the source of this inefficiency, as well as ways in which it can be reduced.

Plots of reduced plate height vs. reduced velocity can be very useful in comparing efficiencies of chromatographic systems. However, an accurate calculation of the number of theoretical plates, N, is necessary for this to be accomplished. The commonly used equation

$$N = 5.54\left(\frac{t_R}{t_{W_{0.5}}}\right)^2 \qquad (3)$$

where t_R is the retention time and $t_{W_{0.5}}$ is the peak width at half height, has been shown to be highly inaccurate for skewed peaks [24]. Foley and Dorsey [25] have recently described a simple yet accurate equation for plate count which corrects for the asymmetry of skewed peaks. This equation

$$N = \frac{41.7(t_R/t_{W_{0.1}})^2}{B/A + 1.25} \tag{4}$$

was used in this work where B/A is the asymmetry ratio measured at 10% peak height [24]. The reduced plate height, h, was calculated by

$$h = \frac{H}{dp} \tag{5}$$

and

$$H = \frac{L}{N} \tag{6}$$

where H is the plate height, L is the column length, and dp is the stationary phase particle diameter. The reduced velocity, ν, was calculated as

$$\nu = \frac{u(dp)}{D_m} \tag{7}$$

where u is the linear velocity and D_m is the diffusion coefficient of the solute in the mobile phase. For the nonsurfactant-containing mobile phase, the diffusion coefficient of acetone was calculated to be approximately

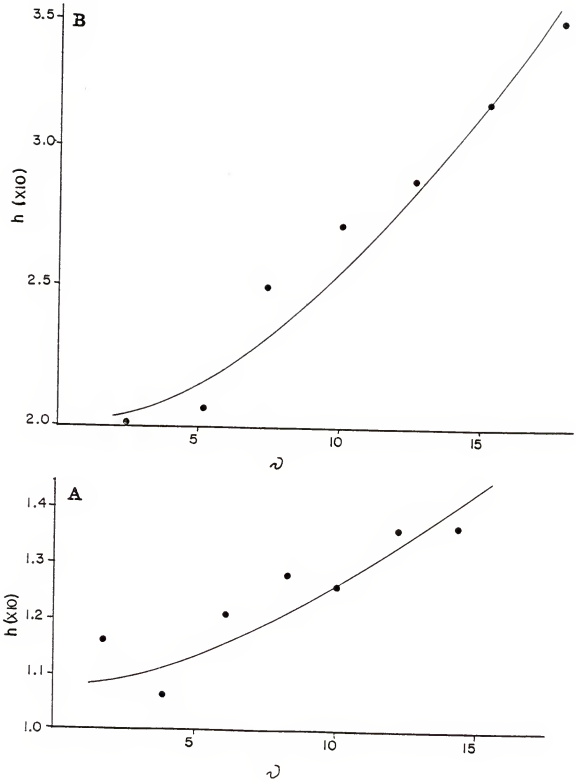
1.3×10^{-5} cm^2/s, estimated by the Wilke-Chang equation [26]. For solutes in micellar mobile phases, the diffusion coefficient is harder to estimate. The diffusion coefficient of SDS in 0.2 M aqueous solution has been reported as about 2×10^{-6} cm^2/s [27]. These values then represent upper and lower limits, as the solute spends some fraction of time in the bulk mobile phase and some fraction of time in the micelle itself. An average value of 7×10^{-6} cm^2/s was then used for these calculations.

Figure 3A is a reduced parameters plot for acetone in a mobile phase of 5/95 methanol/water [28,29]. Figure 3B is for acetone in the same mobile phase with 2×10^{-2} M SDS [28,29]. The steep slope of the plot at high reduced velocities for the micellar system is indicative of high resistance to mass transfer [30]. It is likely that this poor mass transfer is a slow transfer step from the micelle to the stationary phase, as it is well-known that the interior of micelles exhibit somewhat higher viscosity than bulk solution [31].

In a similar analysis of the same data, Figure 4 is a plot of H vs. u. This is included because columns of identical dimensions were used, and reduced parameters (unitless) are often not well understood. The same interpretation of purely aqueous micellar systems having poor mass transfer characteristics can be well noted.

Two approaches have been taken to improve these mass transfer characteristics. Most reports of micellar LC have

Figure 3. Reduced parameter van Deemter plot for acetone: (A) Ultrasphere ODS, mobile phase, 5/95 methanol/water; (B) same as A but mobile phase also 2×10^{-2} M SDS.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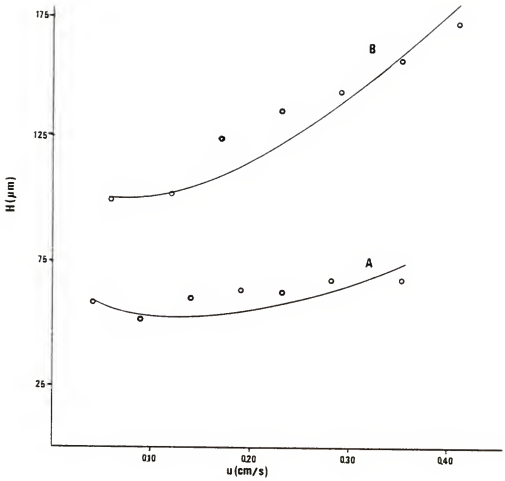


Figure 4. Van Deemter plot for acetone: (A) Ultrasphere ODS, mobile phase, 5/95 methanol/water; (B) same as A but mobile phase also 2×10^{-2} M SDS.

used totally aqueous mobile phases. It is known that hydrophobic stationary phases exhibit very poor mass transfer characteristics under these conditions [32]. This is presumably because of the poor wetting of the stationary phase, resulting in slow equilibrium across the interface of the two highly dissimilar phases. Indeed, when a gradient program with a C_{18} column is run, it has been suggested that the initial aqueous component of the mobile phase contains some minimum concentration of organic solvent to improve wetting of the stationary phase [32]. For this reason, it was felt that the addition of small amounts of an organic solvent to the aqueous micellar mobile phase would greatly improve the efficiency of the system.

Table 1 shows plate counts for a solute of acetone in a mobile phase of 2×10^{-2} M SDS as methanol is added to the mobile phase. While both the symmetry of the peak and the plate count increased dramatically over the range of methanol studied, this certainly does not represent a usable analytical system. It should be noted, however, that these represent true plate counts and would have appeared much higher using the common half-height equation.

Obviously, even better wetting of the stationary phase is necessary. Studies have shown that as much as 40-50% of organic solvent may be required to allow some reversed-phase packings to be operated at highest efficiency [33]. This represents an unrealistic situation for micellar chromatography, so an attempt was made to find an organic solvent

Table 1. Variation of Efficiency and Asymmetry with Methanol Concentration[a]

% Methanol	N	B/A
0	300	6.67
2	350	4.83
5	750	4.00
10	1120	3.50

a Ultrasphere ODS, mobile phase 2×10^{-2} M SDS, acetone solute.

which would give good wetting at low concentrations. Table 2 shows plate counts and asymmetries for benzene in a mobile phase of 5×10^{-2} M SDS in which the 10% organic component was varied. Again, an improvement is seen in both peak symmetry and efficiency as the polarity of the organic modifier is decreased and wetting of the stationary phase is enhanced. Because of the great improvement shown in going from methanol to propanol, a new Altex Ultrasphere ODS column was installed and the 10/90 propanol/water + 5×10^{-2} M SDS experiment was repeated. A plate count of 15,000 with an asymmetry factor of 1.16 was found for benzene at a flow rate of 1.5 mL/min, showing that the data had been limited by the efficiency of the Alltech column. This represents a reduced plate height of 3.3, which is well within the range of efficiencies shown by traditional hydroorganic mobile phases. While the actual values in Table 2 may therefore be low, the trend is still obvious.

A second parameter which can be adjusted to improve mass transfer characteristics is temperature. As the viscosity of a micellar solution will be somewhat greater than that of an identical solution without surfactant, diffusion coefficients will be smaller, thereby slowing mass transfer. Table 3 shows asymmetries and plate counts for benzene in 10/90 propanol/water + 5×10^{-2} M SDS as the temperature is varied from 30 to 50°C. While the efficiency remains virtually the same, an improvement in peak shape is seen at the elevated temperatures. Above a temperature of 40°C, it is necessary

Table 2. Variation of Efficiency and Asymmetry with Organic Modifier[a]

Organic Modifier	N	B/A
methanol	1080	3.51
acetonitrile	1990	1.98
ethanol	2060	2.08
propanol	2230	1.61

a. Alltech C_{18}, 10/90 organic/water + 5×10^{-2} M SDS, benzene solute.

Table 3. Variation of Efficiency and Asymmetry with Temperature[a]

Temp, °C	N	B/A
30	15,000	1.16
35	15,500	1.09
40	14,800	1.06
45	15,000	1.00
50	13,700	1.07

a Ultrasphere ODS, 10/90 propanol/water + 5×10^{-2} M SDS, benzene solute.

to preheat the mobile phase to prevent peak distortion from thermal gradients [34].

It is desirable to keep the concentration of organic modifier as low as possible both to maintain the integrity of the micelle and to minimize expense. Furthermore, micellar chromatography may represent an almost ideal way in which to study certain physical parameters of micelles. As the structure and behavior of micelles are much better understood in purely aqueous systems, added organic modifier can greatly complicate interpretation of data. Table 4 shows asymmetries and plate counts for benzene in 5×10^{-2} M SDS at 40°C as the concentration of propanol is varied. Little improvement is seen above 3% propanol. Scott and Simpson [35] have studied modification of C_{18} phases by organic modifiers and have shown that over 90% of the surface is covered with the alcohol at a concentration of 3% (w/v) propanol, but there is only about 50% coverage with the same concentration of methanol [35]. This modification of the surface allows fast mass transfer, resulting in improved efficiencies. In addition to better wetting of the stationary phase, the added alcohol may be "loosening" the micellar structure so that the local microviscosity experienced by a solubilized solute is diminished. This effect, however, is probably small in comparison to the improved wetting. Addition of low concentrations of organic modifiers also lowers the k' of the test solutes, but this has a negligible effect on the efficiency calculations. Table 4 shows essentially a constant plate count from 3% to

Table 4. Variation of Efficiency and Asymmetry with Propanol Concentration[a]

% Propanol	N	B/A	k'
0	9,070	1.62	23.8
1	14,100	1.15	21.3
2	14,280	1.03	19.6
3	14,540	1.02	18.2
4	15,310	1.00	16.9
6	14,930	1.01	14.9
8	14,710	1.01	13.2
10	15,340	1.01	11.7

a Ultrasphere ODS, mobile phase 5×10^{-2} M SDS, 40°C, benzene solute.

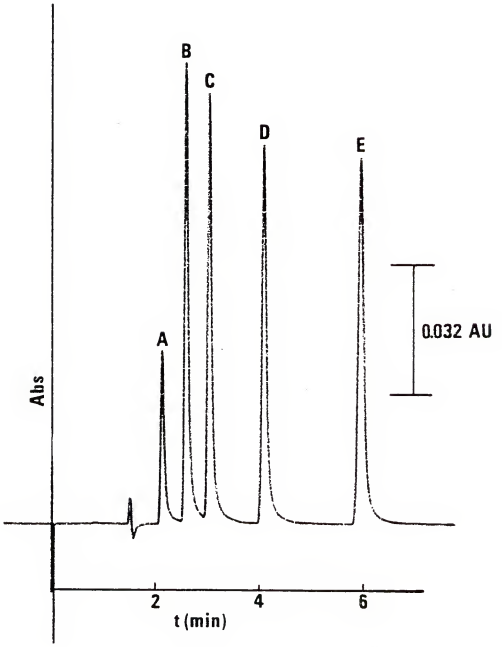
10% added propanol while the k' of benzene was reduced from 18.2 to 11.7 over this range.

Yarmuchuk et al. [36] performed a similar study of micellar efficiency optimization whereby they also suggested higher operating temperatures, but not organic modifiers to improve poor mass transfer. They worked with a C_1 stationary phase so that the major factor in retention was the adsorbed surfactant. Addition of small amounts of organic modifier would displace the surfactant and have a very large effect on retention, while not improving the efficiency significantly.

As seen from Table 4 (0-3% propanol), small amounts of propanol do improve the efficiency significantly while the k' of benzene was reduced from 23.8 to only 18.2. The difference of opinion in the use of organic modifiers for optimization can be resolved by realizing the need for wetting the very hydrophobic C_{18} (and C_8) stationary phases which would be detrimental to the C_1 column, as well as to other slightly polar bonded phase columns.

To fully illustrate the efficiencies attainable with micellar mobile phases, a series of chromatograms was run. Figure 5 is a separation of a five-component mixture using a mobile phase of 70/30 methanol/water. The small amount of tailing on the peaks is from the somewhat slow time constant of the detector used and is only apparent for fast eluting compounds. Figure 6 is the same mixture with a mobile phase of 10/90 propanol/water. The fifth component (toluene) had

Figure 5. Chromatogram of five component test mixture: 25 cm Ultrasphere ODS; mobile phase, 70/30 methanol/water; flow rate, 1.5 mL/min; (A) phenol; (B) acetophenone; (C) nitrobenzene; (D) benzene; (E) toluene.


B
C
D
E
A
0.032 AU
Abs
2
4
6
t (min)

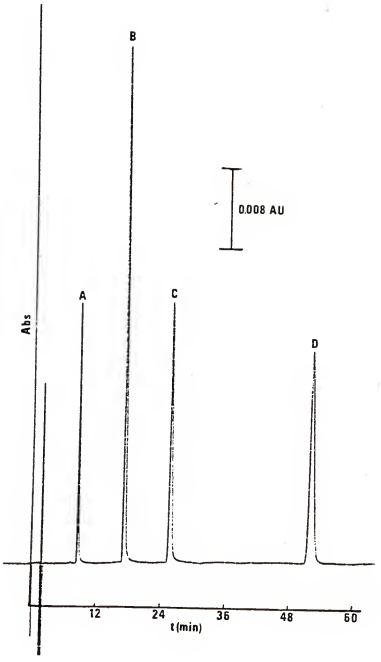


Figure 6. Chromatogram of five component test mixture: same as Figure 5 but mobile phase 10/90 propanol/water.

still not eluted after 2 h. Figure 7 is the same mobile phase but with 5×10^{-2} M SDS added. Toluene now elutes at about 39 min, and all peaks show good symmetry and efficiency. Figure 8 is the same mixture with a mobile phase of 10/90 propanol/water + 1×10^{-1} M SDS. The retention of toluene has been decreased to 24 min. Increasing the concentration of micelles then serves to adjust retention in the same manner as increasing the amount of organic modifier.

Table 5 shows efficiency related data for all 3 surfactants used throughout this study with the "SDS efficiency optimized" conditions (3% propanol and 40°C). Zero percent propanol has been included to show the improvement in the reduced plate height at 3% propanol. For the nonionic surfactant (Brij-35) and the cationic surfactant (CTAB), low reduced plate heights also indicate good mass transfer.

To illustrate the efficiencies and separations possible with the optimized conditions, Figures 9 through 12 are for the 3 surfactants. Figures 9 and 10, using a 25 cm column with a mobile phase containing 5×10^{-2} M SDS and 1×10^{-1} M SDS, respectively, show toluene eluting in 34 and 48 min. Figure 11, using a 15 cm column with 5×10^{-2} M Brij-35, elutes toluene in 20 min, whereas in Figure 12, also using a 15 cm column and 5×10^{-2} M CTAB, toluene elutes in 22 min. An interesting note is the elution order change with CTAB for the solutes phenol (A) and acetophenone (B) from the previous separations.

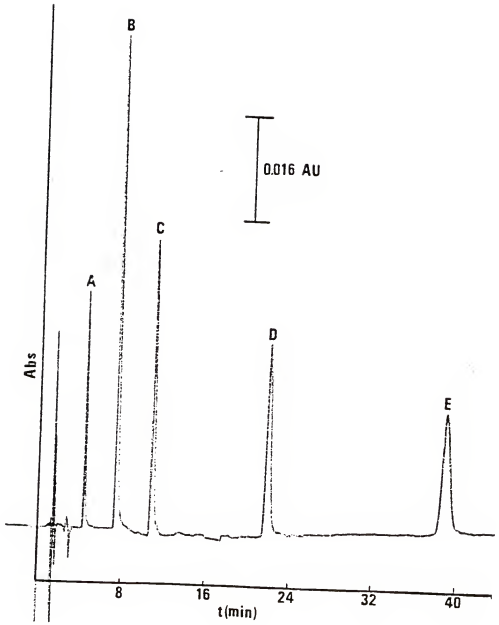


Figure 7. Chromatogram of five component test mixture: same as Figure 5 but mobile phase 10/90 propanol/ water + 5×10^{-2} M SDS.

Figure 8. Chromatogram of five component test mixture: same as Figure 5 but mobile phase 10/90 propanol/water + 1×10^{-2} M SDS.

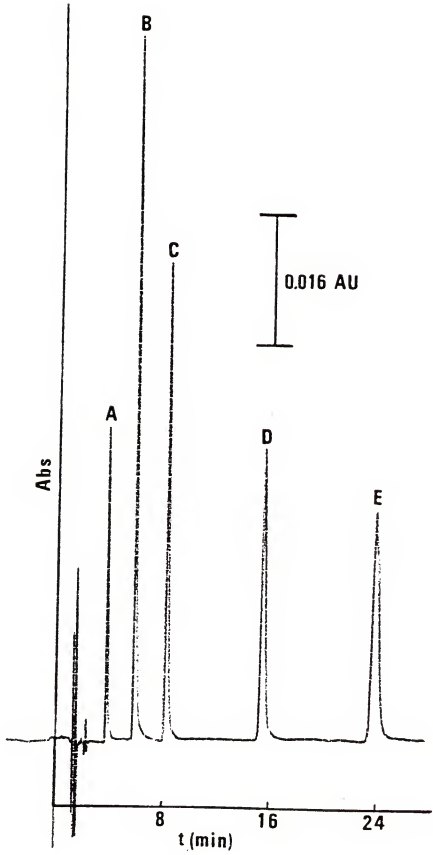

B
C
0.016 AU
A
D
E
Abs
8
16
24
t (min)

Table 5. Efficiency Data vs. Surfactant for "Optimized" Conditions[a]

	% Propanol	Surfactant	h	L(cm)	N	B/A
a	0	5×10^{-2} M SDS	5.5	25	9,070	1.62
b	3	5×10^{-2} M SDS	3.4	25	14,540	1.02
c	3	5×10^{-2} M Brij-35	4.1	15	7,240	1.03
d	3	5×10^{-2} M CTAB	4.1	15	7,290	1.04

a Altex Ultrasphere ODS, 40°C, benzene solute.
Columns a and b were the same.
Columns c and d were different.

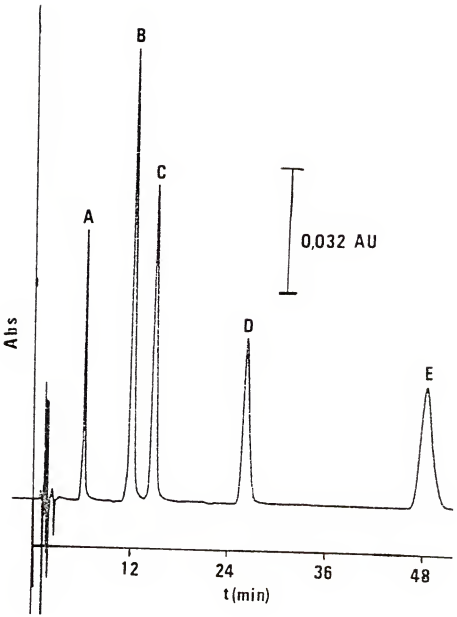


Figure 9. Chromatogram of five component test mixture: same as Figure 5 but mobile phase 3/97 propanol/ water + 5×10^{-2} M SDS.

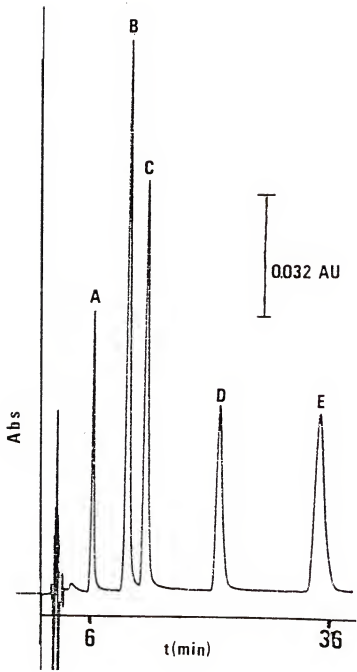


Figure 10. Chromatogram of five component test mixture: same as Figure 5 but mobile phase 3/97 propanol/water + 1×10^{-1} M SDS.

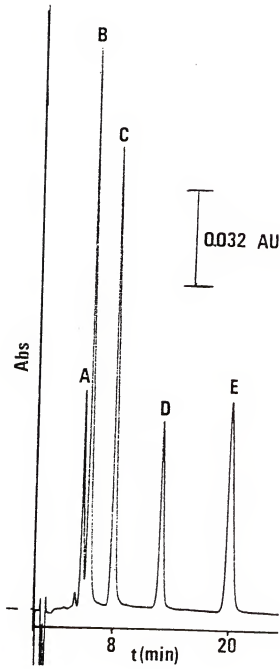


Figure 11. Chromatogram of five component test mixture: same as Figure 5 but 15 cm Ultrasphere ODS and mobile phase 3/97 propanol/water + 5×10^{-2} M Brij-35.

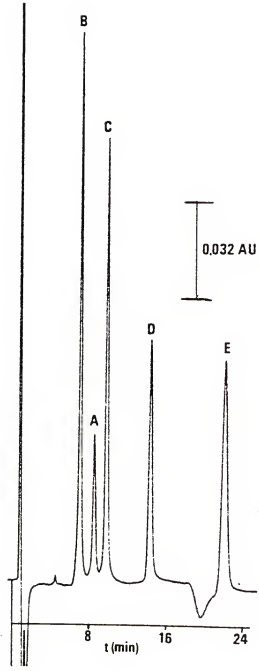


Figure 12. Chromatogram of five component test mixture: same as Figure 5 but 15 cm Ultrasphere ODS and mobile phase 3/97 propanol/water + 5×10^{-2} M CTAB.

CHAPTER IV

CHARACTERIZATION

The fundamental parameter for comparison of retention data and interpretation of physical phenomena is the capacity factor, k'. The capacity factor expresses the length of time the solute is retained by the stationary phase relative to the time spent in the mobile phase and is easily calculated by equation (8).

$$k' = \frac{t_r - t_0}{t_0} \tag{8}$$

where t_r is the retention time of a retained solute and t_0 is the hold-up time or time for an unretained solute to elute. The void volume, V_0, is then related to t_0 by equation (9).

$$V_0 = t_0 F \tag{9}$$

where F is the volume flow rate.

Void Volume Determination

An accurate determination of column void volume is essential for meaningful capacity factor calculations. This problem has received some attention in the literature [37-40],

but there is yet to be a consensus on a correct approach. The use of $NaNO_3$ gives a very reproducible value, is unretained by a hydrophobic stationary phase, and is small enough to be accessible to the pores of the silica [39]. This method was used for nonsurfactant-containing mobile phases but, because of electrostatic effects, will not work for mobile phases containing ionic surfactants. For surfactant-containing mobile phases, the refractive index change from an injection of water gave comparable values to those determined with nitrate in nonsurfactant-containing mobile phases. An approximately 2.4 mL volume was used for the 25 cm column while 1.4 mL was used for the 15 cm column for all k' determinations.

Thermodynamics

The effect of temperature on retention is related to the enthalpy of transfer of the solute from the mobile phase to the stationary phase [41]. A study of the thermodynamics of transfer for a given solute gives some insight into the retention mechanism and helps in understanding the system as a whole.

For a chromatographic process, a van't Hoff plot is usually constructed. With a plot of log k' vs. 1/T, the standard enthalpies and entropies of transfer can be calculated. The capacity factor, k', is related to the thermodynamic distribution constant by

$$k' = \phi K \qquad (10)$$

where ϕ is the volume phase ratio (stationary/mobile). Two expressions of the Gibbs free energy are

$$\Delta G° = -RT \ln K \quad (11)$$

and

$$\Delta G° = \Delta H° - T\Delta S° \quad (12)$$

Combining these three equations, an expression for the capacity factor in terms of the thermodynamic properties $\Delta H°$ and $\Delta S°$ is obtained.

$$\ln k' = \frac{-\Delta H°}{RT} + \frac{\Delta S°}{R} + \ln \phi \quad (13)$$

If $\Delta H°$ and $\Delta S°$ are independent of temperature over the temperature interval studied, the van't Hoff plots will be linear. An unchanging slope will give a constant enthalpy of transfer, which can be interpreted as an unchanging retention mechanism over that temperature range.

A van't Hoff plot was constructed for anisole over a temperature range of 30-50°C in 5°C intervals, with a mobile phase of 10/90 methanol/water. This plot shows excellent linearity (r = 0.998) and yields a $\Delta H°$ value of -4.7 kcal. Another van't Hoff plot was constructed for anisole over the same temperature range with a mobile phase of 10/90 methanol/water + 2×10^{-2} M SDS, which is safely above the CMC of 7.9×10^{-3} M reported for SDS in a 12.6% methanol solution [42]. This plot also shows excellent linearity (r = 0.997) and yields a $\Delta H°$ value of -2.1 kcal. The range of enthalpy values found for a variety of compounds is in agreement with the values expected for a reversed phase system, typically

from -1 to -10 kcal/mol [43], and the values are shown in Table 6. The large negative values of $\Delta H°$ of transfer are observed for the solutes from a micellar-free mobile phase to a micellar one, showing the ability of the micelle to compete with the stationary phase for the solute. The linearity of the van't Hoff plot for the micellar system is also evidence that the integrity of the micelle structure is maintained over the temperature range investigated. This is an important consideration as temperature changes can affect the aggregation number and shape of micellar structures [44].

Electrolyte and Micellar Shape

Ever since the conclusive evidence for the existence of micelles [45], there has been much controversy over the shapes of micellar structures. It is generally accepted that small micelles are spherical in nature [1]. Both the CMC and aggregation number can be affected to varying extentes by any number of factors (chain length, pressure, temperature, co-solvents, surfactant concentration, electrolytes, etc.), and it is theorized that as micellar size increases, spherical shapes are not always feasible and a transition to rod-like structures has been suggested [46].

The addition of a small electrolyte (i.e., NaCl) has been argued by many [47-51] to cause the transition of SDS micelles from spherical to rod-like at 0.45 M NaCl. A recent study [51] using a spectroscopic probe (ε_τ 30) has shown micellar polarity starting to decrease in a solution

Table 6. $\Delta H°$ of Transfer[a] [28,29]

	$\Delta H°$, kcal/mol	
	no SDS	2×10^{-2} M SDS
Acetone	-2.28	
MEK	-2.99	-1.17
Benzene	-3.13	-1.59
Anisole	-4.68	-2.06

a Ultrasphere ODS, 10/90 methanol/water

of 0.2 M NaCl, but as of yet there has been no independent confirmation of this. Spectroscopic techniques are currently the most widely used analytical methods for investigating micellar structure [2], but subtle polarity changes are often difficult to see. Since polarity controls the strength of a reversed phase mobile phase, LC may be a nearly ideal method for studying changes in micelle polarity.

By measuring a test solute's retention (k'), as a function of electrolyte concentration using a purely aqueous micellar mobile phase, any change in polarity of the micelle (e.g., spherical to rod-like) should manifest itself in a dramatic change in retention. A C_1 column was chosen over longer alkyl chain columns because its smaller organic moiety eliminates changes in conformation of the alkyl chains as electrolyte is added which would mask any concomitant retention effects. Toluene was chosen as the test solute because of its nonpolar nature and ability to partition into (or onto) the micelle, giving a reasonable retention time.

Figure 13 is a plot of k' for toluene on the C_1 column at 25°C vs. NaCl concentration (in moles/liter) with a constant concentration of 2.7×10^{-2} M SDS. At first, it appears as though the addition of salt is simply changing the surface tension resulting in an increased k' [52], as is also occurring with the non-SDS mobile phase (blank).

However, if the first derivative (with blank subtracted) is taken for the SDS containing mobile phase, Figure 14

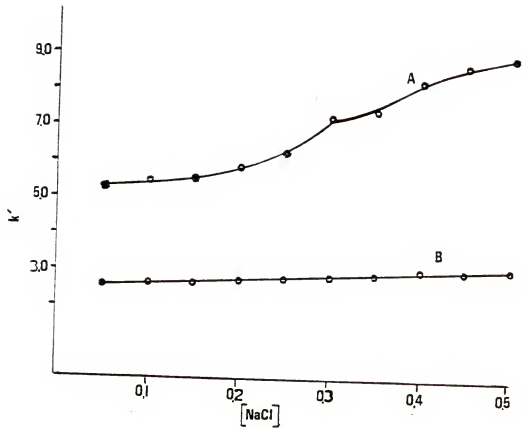
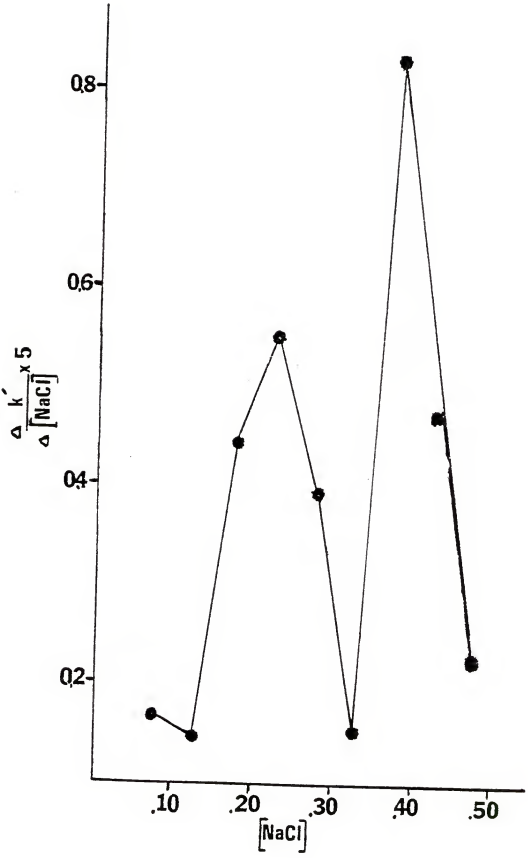


Figure 13. Capacity factor vs. NaCl concentration at 25°C for toluene on C_1 mini column, with 2.5×10^{-2} M SDS (A); and without SDS, blank (B).

Figure 14. First derivative of capacity factor vs. NaCl concentration at 25°C for toluene on C_1 mini column for 2.5×10^{-2} M SDS with blank subtracted.


0.8
0.6
0.4
0.2
$\frac{\Delta k'}{\Delta [NaCl]} \times 5$
.10
.20
.30
.40
.50
[NaCl]

(with data points connected for clarity) demonstrates the curious results of 2 maxima at 0.225 M and 0.375 M NaCl. These values could correspond to the 0.2 M and 0.45 M transitions of previous studies (vide supra).

In a similar treatment, Figure 15 is a plot of k' for benzene on a cyano column at 25°C vs. NaCl concentration with a constant concentration of 2.5×10^{-2} M SDS. However, Figure 16 demonstrates only one maximum (at 0.325 M NaCl). At this point, no conclusions can be drawn from this preliminary work, and future work is necessary.

CMC Determination and Effect of Surfactant Concentration

From the IUPAC Manual of Symbols and Terminology [53], the following statement has been taken:

> "There is a relatively small range of concentrations separating the limit between which virtually no micelles are detected and the limit above which virtually all additional surfactant forms micelles. Many properties of surfactant solutions, if plotted against the concentration, appear to change above and below this range. By extrapolating the loci above and below this range until they intersect, a value may be obtained known as the critical micellization concentration..." (CMC).

Some experimental techniques utilized to determine CMC's include electrical conductance, surface tension, turbidity, solubilization of compounds of low water solubility, osmotic pressure, and NMR [2]. Figure 17 is a plot of log k' vs. log SDS concentration (in moles/liter) for nitrobenzene and benzene in a mobile phase of 10/90 1-propanol/water. Two linear

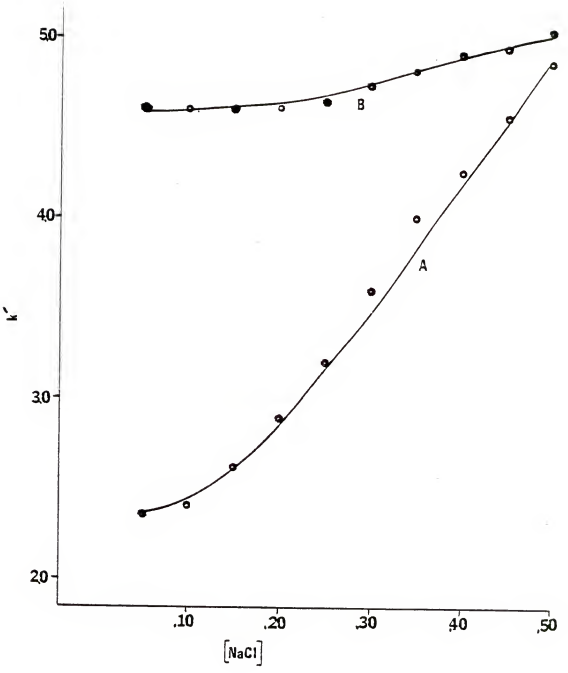


Figure 15. Capacity factor vs. NaCl concentration at 25°C for benzene on cyano column, with 2.7×10^{-2} M SDS (A); and without any SDS, blank (B).

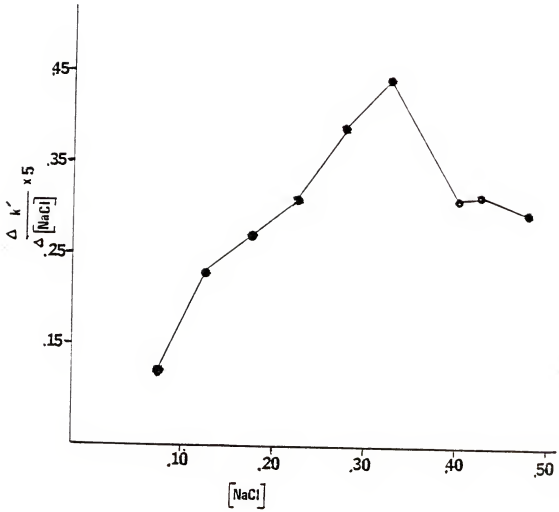


Figure 16. First derivative of capacity factor vs. NaCl concentration at 25°C for benzene on cyano column for 2.7×10^{-2} M SDS with blank subtracted.

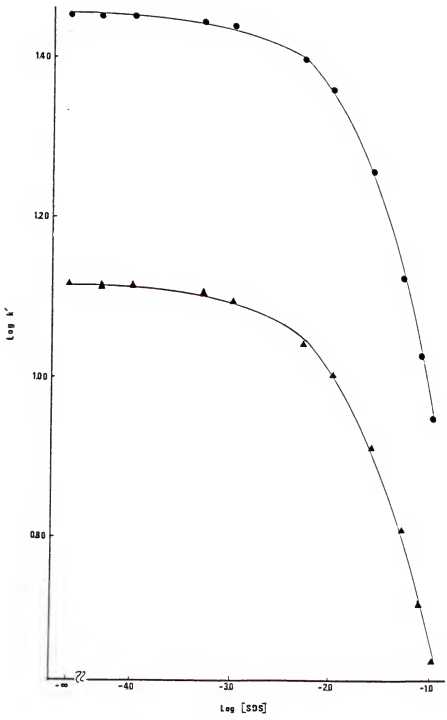


Figure 17. Log k' vs. log[SDS]: Ultrasphere ODS, mobile phase, 10/90 propanol/water; flow rate, 1.5 mL/min; (●) benzene; (▲) nitrobenzene.

components of these plots are seen, one above and one below the critical micelle concentration (CMC). Curvature occurs in the region of the CMC, but extrapolation of the two linear portions should show an intersection at approximately the CMC of the particular system under investigation. The CMC of SDS in pure water at 25°C is 8×10^{-3} M and has been reported to first decrease and then increase with small additions of methanol [42]. The value calculated from Figure 17 is 0.013 M from both nitrobenzene and benzene data in the system of 10/90 1-propanol/water at 40°C. The effect of the cosolvent on the micelle structure is then an important consideration. In solutions of 0.27 mole fraction methanol, micellar aggregation of SDS molecules is precluded [42]. For this and other reasons, it is important that the concentration of the cosolvent be kept as low as possible.

The slopes for both components are slightly negative for concentrations of SDS below the CMC. Other authors also have reported small decreases in k' for nonionic solutes as SDS concentration is increased [19,54]. Knox and Hartwick [54] have shown that SDS adsorbs almost irreversibly onto a C_{18} phase, and it is likely that adsorbed SDS remained on the column from previous experiments. The linear portion of the plot for concentrations above the CMC is evidence that the system is behaving in a well-defined reversed-phase manner and that the micelle concentration controls retention of these solutes in the same way as do organic modifiers [55].

Figures 18-20 are log k' vs. log [surfactant] concentration plots for SDS, CTAB and Brij-35, respectively, for the

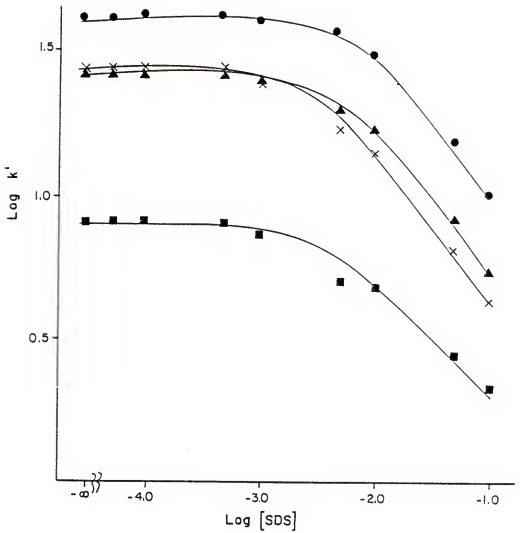


Figure 18. Log k' vs. log[SDS]: Ultrasphere ODS; mobile phase, 3/97 propanol/water; flow rate, 1.5 mL/min; (•) benzene; (▲) nitrobenzene, (×) acetophenone; (■) phenol.

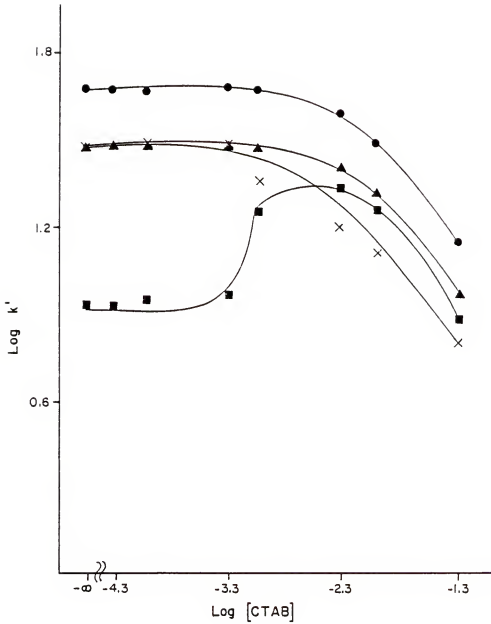
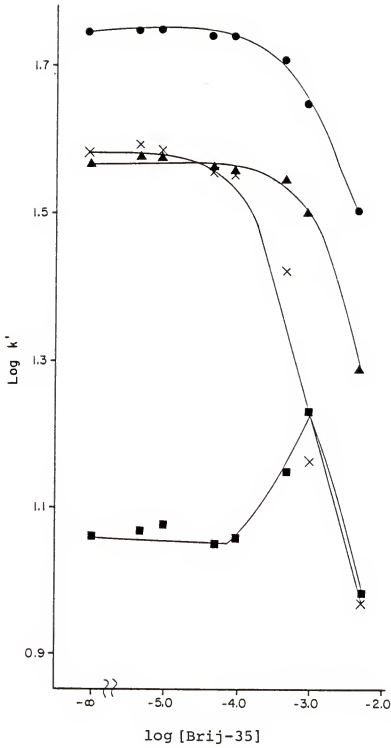


Figure 19. Log k' vs. log [CTAB]: Ultrasphere ODS; mobile phase, 3/97 propanol/water; flow rate 1.5 mL/min; (●) benzene; (▲) nitrobenzene; (×) acetophenone; (■) phenol.

Figure 20. Log k' vs. log[Brij-35]: Ultrasphere ODS; mobile phase, 3/97 propanol/water; flow rate, 1.5 mL/min; (●) benzene; (▲) nitrobenzene; (✖) acetophenone; (■) phenol.


1.7
1.5
1.3
1.1
0.9
Log k'
-∞
-5.0
-4.0
-3.0
-2.0
log [Brij-35]

optimized conditions (see Chapter III). The CMC values calculated from the above figures using the solutes nitrobenzene and benzene are shown in Table 7 and compared to literature values. Although CMC temperature dependence is complex with minima generally occurring, it is overall a small variation [1,2]. Organic cosolvents can be incorporated into the micelle reducing the CMC, or can modify solvent-surfactant interactions increasing the CMC, or can do either depending on cosolvent concentration [1,2]. Therefore, both temperature and solvent differences can explain the slight variations in the chromatographic method from the literature values cited. Notice as expected, the nonionic CMC is much smaller than either of the ionic sufactants [1].

In Figures 19 and 20, CTAB and Brij-35, respectively, notice the unusual or at least very differently shaped phenol curves with maximas near the CMC's. The fact that this occurs for both the cationic and nonionic surfactants and not the anionic surfactant (Figure 18) indicates an intriguing and as of yet unexplained retention mechanism. The other solutes studied behave in a similar and expected manner for all 3 surfactants (vide supra). As a point of reference to traditional hydroorganic mobile phases, Figures 21 and 22 are plots of log k' vs. percent methanol, and log k' vs. log percent MeOH, respectively.

Table 7. CMC Values

Surfactant	Chromatographic Method	Literature
SDS	1.3 E - 2 M[a] 1.3 E - 2 M[b]	8.0 E - 3 M[c]
SDS	4.2 E - 3 M[d] 5.3 E 3 M[e]	8.6 E - 3 M[f]
CTAB	3.8 E - M[g] 3.4 E - 3 M[h]	9.2 E - 4 M[i]
	3.2 E - 4 M[j] 3.6 E - 4 M[k]	6.0 E - 5 M[l] 3.0 E - 5 M[m]

	Solute or Scientist	T (°C)	Solvent
a	Nitrobenzene	T = 40°C	10/90 PrOH/H_2O
b	Benzene	T = 40°C	10/90 PrOH/H_2O
c	Flockhart, 1961†	T = 25°C	100% H_2O
d	Nitrobenzene	T = 40°C	3/97 PrOH/H_2O
e	Benzene	T = 40°C	3/97 PrOH/H_2O
f	Flockhart, 1961†	T = 40°C	100% H_2O
g	Nitrobenzene	T = 40°C	3/97 PrOH/H_2O
h	Benzene	T = 40°C	3/97 PrOH/H_2O
i	Czerniawski, 1966†	T = 25°C	100% H_2O
j	Nitrobenzene	T = 40°C	3/97 PrOH/H_2O
k	Benzene	T = 40°C	3/97 PrOH/H_2O
l	Schick, 1965†	T = 25°C	100% H_2O
m	Schick, 1965†	T = 55°C	100% H_2O

† Taken from Reference [1].

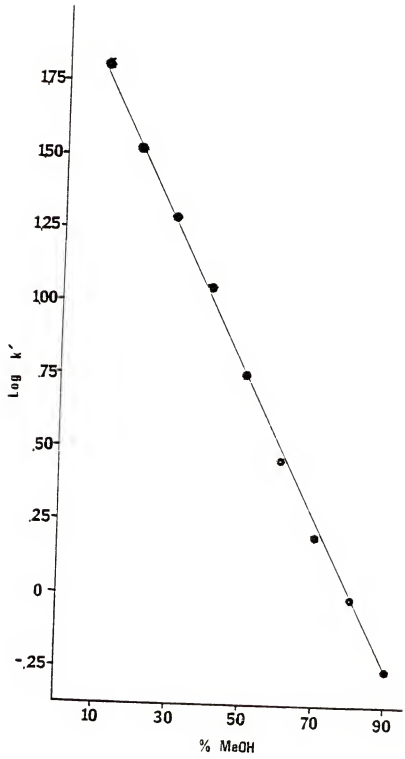


Figure 21. Log k' vs. % methanol: Ultrasphere ODS; mobile phase, methanol/water; flow rate, 1.5 mL/min for benzene.

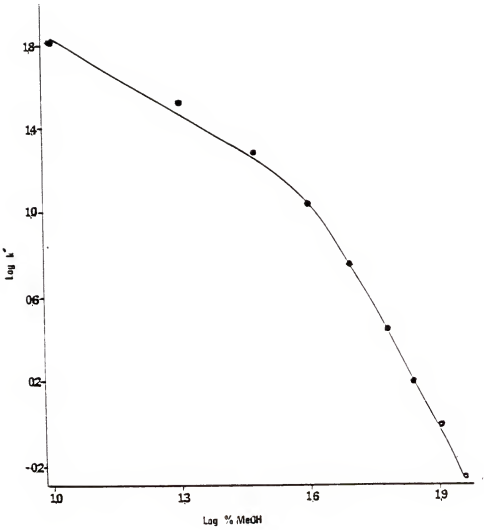


Figure 22. Log k' vs. log % methanol: Ultrasphere ODS; mobile phase, methanol/water; flow rate, 1.5 mL/min for benzene.

Selectivity

The ratio of two capacity factors is the separation selectivity, α, which describes the relative retention of two solutes, an important parameter to adjust when resolving closely eluting peaks. From the relation

$$\alpha = k'_2/k'_1 \tag{14}$$

it can be deduced that

$$\log \alpha = |\log k'_2 - \log k'_1| = \Delta \tag{15}$$

The value Δ is then directly related proportionally to the selectivity. Obviously, when this value is zero, there is complete overlap of peaks. A way of observing the selectivity change for a pair of solutes as the concentration of modifier is added is to plot log k' vs. log[surfactant]. In Figure 18, there is little or no separation ($\Delta \approx 0$) of acetophenone and nitrobenzene below the CMC, but Δ increases as the surfactant concentration increases above the CMC.

A more conventional method of describing selectivity changes for the three surfactants is shown in Figures 23 through 28. These selectivity vs. log[surfactant] plots should be considered along with the log k' vs. log[surfactant] plot (Figures 18-20). These six selectivity plots are the possible permutations of the four solutes, for each surfactant. Consistent among the plots is the essentially consistent α below the CMC. Figures 23-27 demonstrate very significant changes above the CMC.

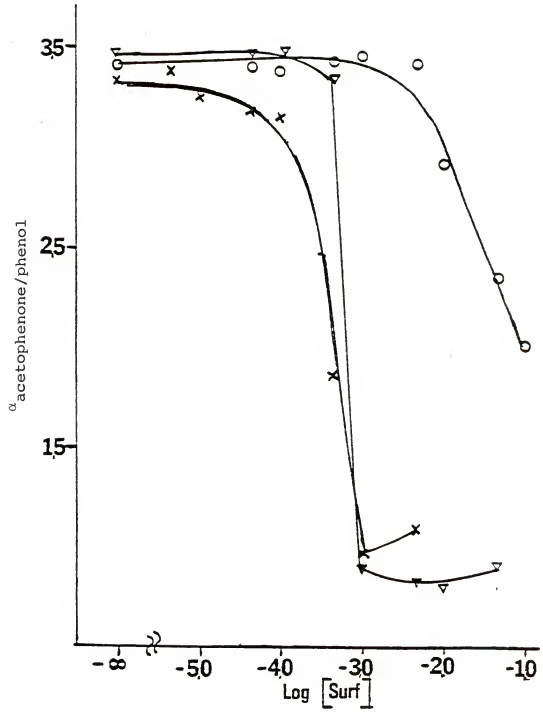


Figure 23. Selectivity of acetophenone/phenol vs. log[surfactant]: Ultrasphere ODS; mobile phase, 3/97 propanol/water; and surfactants (o) SDS, (Δ) CTAB, (X) Brij-35.

Figure 24. Selectivity of nitrobenzene/phenol vs. log[surfactant]: same conditions and symbols as Figure 23.

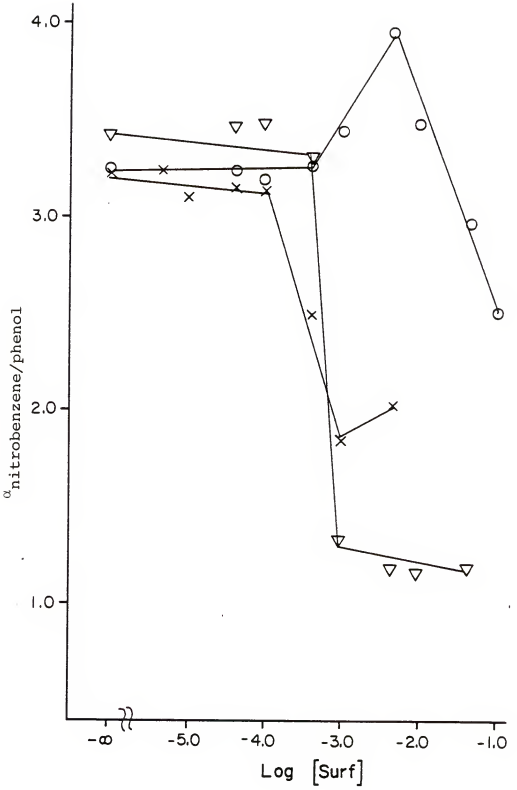
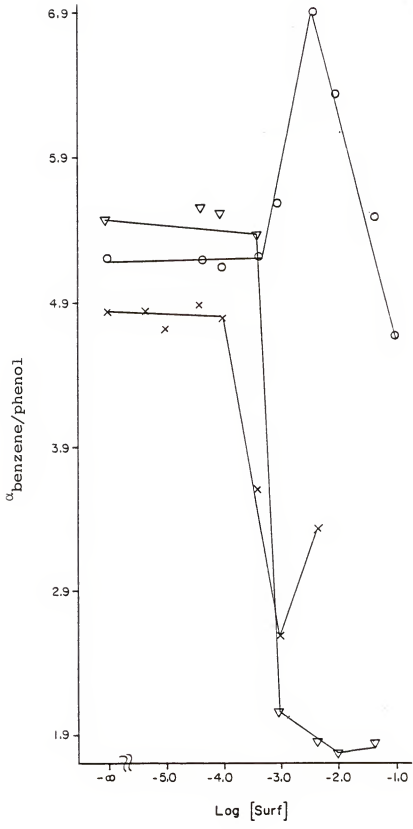

4.0
3.0
2.0
1.0
$\alpha_{\text{nitrobenzene/phenol}}$
-∞
-5.0
-4.0
-3.0
-2.0
-1.0
Log [Surf]

Figure 25. Selectivity of benzene/phenol vs. log[surfactant]: same conditions and symbols as Figure 23.


6.9
5.9
4.9
3.9
2.9
1.9
$\alpha_{benzene/phenol}$
-∞
-5.0
-4.0
-3.0
-2.0
-1.0
Log [Surf]

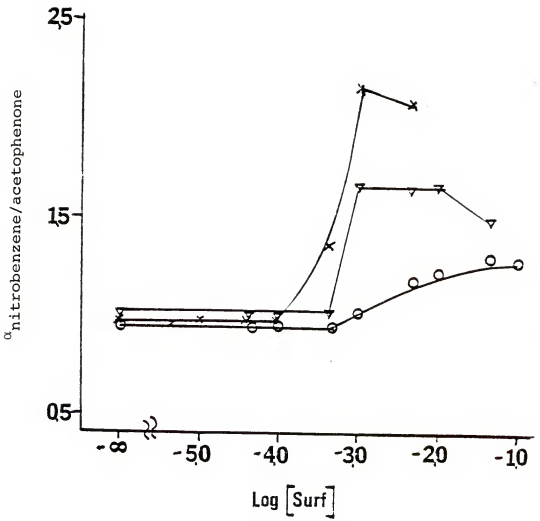
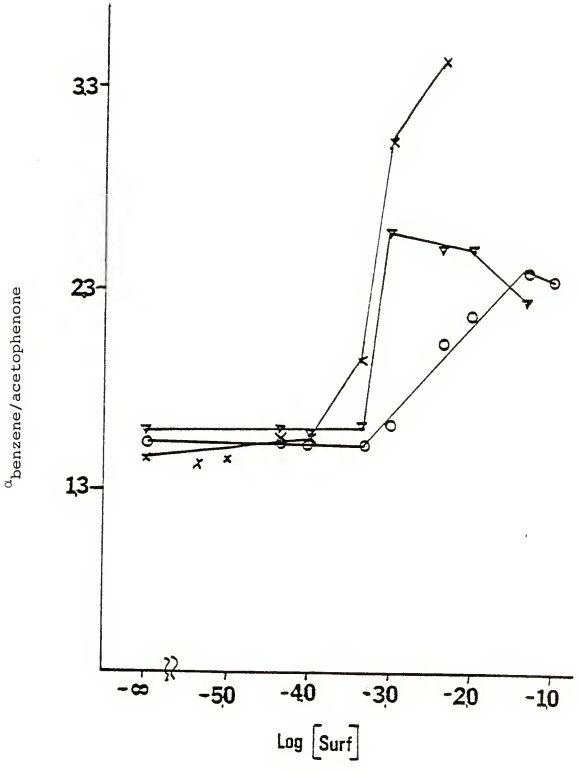


Figure 26. Selectivity of nitrobenzene/acetophenone vs. log[surfactant]: same conditions and symbols as Figure 23.

Figure 27. Selectivity of benzene/acetophenone vs. log[surfactant]: same conditions and symbols as Figure 23.


33
23
13
$\alpha_{benzene/acetophenone}$
-8
-50
-40
-30
-20
-10
Log [Surf]

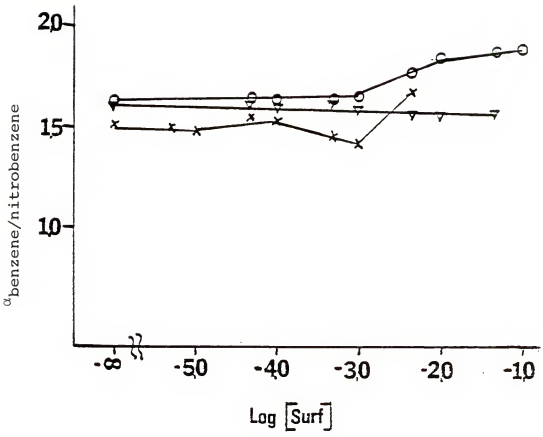


Figure 28. Selectivity of benzene/nitrobenzene vs. log[surfactant]: same conditions and symbols as Figure 23.

The dramatic decrease in selectivity for acetophenone and phenol ($\alpha_{acetophenone/phenol}$) in Figure 23 for both CTAB and Brij-35 is due to simultaneous increased phenol retention and decreased acetophenone retention to the point of elution order reversals ($\alpha < 1$). For SDS, the decrease in selectivity is due to the steeper slope for acetophenone relative to phenol as can be seen in the log k' vs. log[SDS] plot (Figure 18).

Figures 26 and 27 show increases in selectivity for nitrobenzene/acetophenone and benzene/acetophenone. As seen in Figures 18-20, all solutes show decreased retention, however, the slopes for acetophenone are greater than either nitrobenzene or benzene, especially for Brij-35. The steeper slopes indicate greater partitioning of solute from bulk mobile phase to micelle with eventual elution order reversals as micelle concentration is increased.

Electrostatic Effects

To examine the electrostatic effects of the ionic surfactants, two ionic solutes, anilinium chloride (An^+) and sodium benzoate (BZ^-) were chosen. Numerous results and mechanisms can be seen from the ionic solute plot of log k' vs. log[surfactant] (Figure 29). The solid line represents the cationic surfactant, CTAB, while the dotted line represents the anionic surfactant, SDS.

As expected with no surfactant (log[surfactant] $\approx -\infty$), the cation and anion have little or no retention. However,

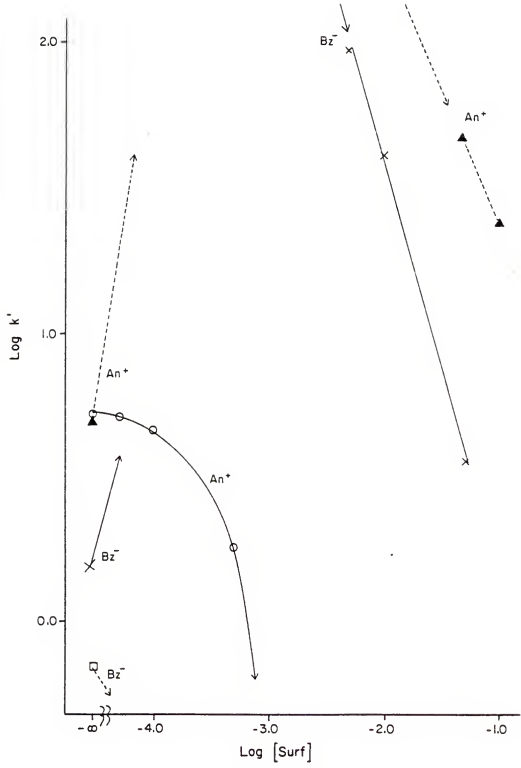


Figure 29. Log k' vs. log[ionic surfactant] for anilinium chloride (▲) and sodium benzoate (○): Ultrasphere ODS; flow rate, 1.5 mL/min; mobile phase, 3/97 propanol/water + SDS (···) or CTAB (—). Arrows show trend of points not included on graph.

as the surfactant concentration is increased (still below the CMC), the much studied, if not well understood, mechanism [56-64] of ion-pair chromatography dominates. The ionic solutes form ion-pairs with the oppositely charged ionic surfactants:

$$An^+ + SDS^- = An - SDS \quad (16)$$

$$Bz^- + CTAB^+ = Bz - CTAB \quad (17)$$

with resultant long retention times characteristic of such big hydrophobic solutes. At the same concentrations (below the CMC), it can be seen that solute and surfactant of the same charge do not form ion-pairs, and increasing the surfactant concentration serves only to decrease retention of already poorly retained ionic solutes.

Above the CMC, because of the large concentration of micelles present, the ion-pairs An-SDS and Bz-CTAB elute much faster, typical of non-polar solutes in micellar mobile phases. The combined effect of ion-pairing and partitioning of the ion-pair into the micelle poses a unique way of separating ionic and nonionic species simultaneously and also may give an explanation for the unusual maxima seen for phenol with CTAB (Figure 19).

Gradient Elution

One of the most powerful techniques available to chromatographers involves increasing the strength of the mobile phase during the course of a particular separation.

Gradient elution accelerates late eluting solutes while early, closely eluting peaks remain well resolved. The most serious drawback of gradient elution is the reequilibration time of the column to initial conditions. After a gradient program in reversed phase LC, as much as twenty column volumes of mobile phase can be required to reequilibrate the column to the initial condition. As shown by Scott and Kucera [33,65], McCormick and Karger [37], and Slaats et al. [66], a significant amount of the organic solvent is extracted by the stationary bonded-reverse phase. Gilpin et al. [67] have shown that after conditioning with organic solvent and subsequent washing with copious amounts of pure water, entrapped organic solvent elutes for many column volumes. Therefore, despite the faster gradient separation, reequilibration time spent can be so prohibitive that even a very long isocratic analysis will often be tolerated. Although the mechanism is very different from reversed phase liquid chromatography, a report of a micellar gradient for size exclusion chromatography of polymers has been shown [68].

Figure 30 demonstrates a separation of some small aromatic hydrocarbons [phenol (A), acetophenone (B), nitrobenzene (C), benzene (D), toluene (E), ethylbenzene (F), and anthracene (G)] using a 0.05 M SDS (above CMC) mobile phase. Anthracene (G) does not elute for over 2 hr, however, the early eluting compounds are certainly well resolved. Figure 31 shows the same mixture separated with a much

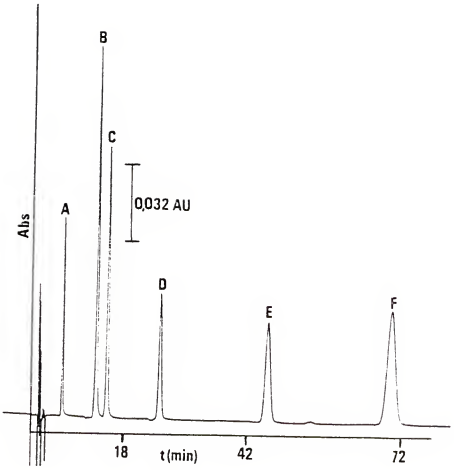


Figure 30. Chromatogram of seven component test mixture: 25 cm Ultrasphere ODS; mobile phase, 3/97 propanol/water + 5×10^{-2} M SDS; flow rate, 1.5 mL/min; (A) phenol; (B) acetophenone; (C) nitrobenzene; (D) benzene; (E) toluene; (F) ethylbenzene, and not shown (G) anthracene.

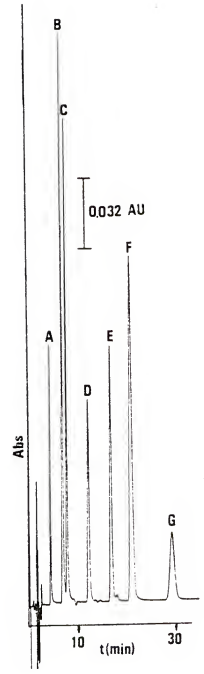


Figure 31. Chromatogram of seven component test mixture: same as Figure 30 but mobile phase, 3/97 propanol/water + 2×10^{-1} M SDS and (G) anthracene shown.

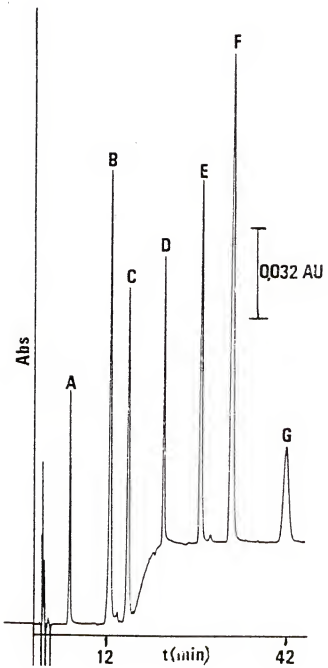


Figure 32. Chromatogram of seven component test mixture: same as Figure 30 but mobile phase, 3/97 propanol/water + SDS gradient program; 5×10^{-2} M SDS initial condition at time 12 min ramp in 2 min to final condition, 2×10^{-1} M SDS and hold; and (G) anthracene shown.

stronger (0.20 M SDS) mobile phase; anthracene elutes in 30 min, however, the early peaks, though not poorly, are not well resolved. Figure 32 shows a gradient micellar separation. After 12 min, a gradient from 0.05 M to 0.20 M SDS over 2 min was made. The early eluting peaks remain well resolved while the last solute, anthracene (G), elutes in just 42 min. The shift in baseline of about 0.032 absorbance units full scale (AUFS) is due to a change in the refractive index of the two micellar solutions. More advanced model UV detectors would more than likely be able to compensate for such changes in the refractive index, reducing the baseline shift. Although a more difficult separation involving a mixture of more closely eluting peaks would better exemplify the technique, the feasibility of micellar gradients has been estabilished for reversed phase LC.

Perhaps as compelling as establishing micellar gradients as a viable technique are the rapid reequilibrations to the initial conditions that are possible. It is believed that starting above the CMC, no further surfactant can adsorb onto the stationary phase as the surfactant concentration is increased (strengthening the mobile phase), and none will desorb when returning to initial conditions because the concentration of free monomer surfactant is constant as long as micelles are present. The advantage over traditional hydroorganic mobile phases in solvent consumption and overall analysis time, including reequilibration times, could be significant when comparing isocratic and gradient analyses.

CHAPTER V

CONCLUSION AND SUGGESTIONS FOR FUTURE WORK

Micellar mobile phases have been characterized in terms of efficiency optimization, some physical studies possible, selectivity, and the capability of gradient elution. Before micellar chromatography with its unique advantages can be accepted as a viable technique, it must first have efficiencies equivalent to traditional hydroorganic mobile phases. The source of purely aqueous micellar mobile phase inefficiency has been attributed to poor wetting of the stationary phase. It has been demonstrated that micellar mobile phases require about 3% propanol and temperatures of about 40°C to attain efficiencies attainable with hydroorganic mobile phases when using very hydrophobic stationary phases.

Van't Hoff plots have been used to calculate heats of transfer, demonstrating the ability of the micelle to compete with the stationary phase for a solute. Critical micelle concentrations have been determined chromatographically for an anionic, a cationic, and a nonionic surfactant, and favorably compared to literature values. Liquid chromatography also has been suggested as a sensitive tool to study structural changes of micelles as a function of electrolyte addition. The results of this study of micellar structure

are only preliminary, but this technique shows promise and should be further investigated.

Unique selectivities of micellar mobile phases have been shown and contrasted for small aromatic hydrocarbons. Also, electrostatic effects for ionic surfactants were demonstrated using two oppositely charged ionic solutes. Besides the efficiency optimization, perhaps the most significant aspect of this work has been the demonstration of micellar gradient elution capabilities. Micellar gradients allow the strength of a mobile phase to be changed very rapidly, leading to fast reequilibration times, not associated with traditional hydroorganic mobile phases.

Of paramount importance in any retention measurement is the determination of a column's void volume. If above the CMC additional surfactant adsorbs to the stationary phase, a column's void-volume could be decreased. Therefore, adsorption isotherms of surfactant solutions should be studied above the CMC. Fast reequilibration could also be explained for micellar gradient chromatography if there is no desorption of surfactant from the stationary phase above the CMC.

A systematic study of selectivity is a necessity. Surfactants varying in chain length, head groups, and counterions should be studied to get a fundamental grasp of the subject before surfactants with exotic functionalities are used. This study has only shown one anionic, cationic and nonionic surfactant, but Zwitterionic and dipolar surfactants are available and should show unusual effects.

Since each ionic surfactant studied can separate oppositely charged solutes in addition to nonionic solutes, perhaps Zwitterionic surfactants will be the answer to separating all types of species. An obvious possibility will be to eventually combine different surfactants forming mixed micelles with intermediate properties.

A problem of ion-pair and probably, therefore, micellar chromatography is irreproducibility. It would be interesting to study the effect of precolumns on the lifetime of analytical columns with a day-to-day comparison of capacity factors of some test solute. It has been mentioned that surfactant preparations vary from lot to lot, and this would also be of practical interest for chromatographers to study.

Finally, a comment on the future of micellar chromatography is in order. Micellar chromatography is still in its infancy and should not yet be too harshly judged. This relatively new technique probably will not replace traditional hydroorganic mobile phases, but because of certain advantages, including the unique selectivities, many practical applications (possibly protein separations) will certainly follow.

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BIOGRAPHICAL SKETCH

John Steven Landy was born on February 11, 1957, in Manhasset, New York. He attended the East Meadow public school system. He received a B. A. degree in chemistry from New York University in 1979. He went directly to the University of Florida where he received the M. S. degree in analytical chemistry in 1982.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. John G. Dorsey, Chairman
Assistant Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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Graduate Research Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the Department of Chemistry in the College of Liberal Arts and Sciences and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 1983

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